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APPLICATION NUMBER: *60/555,797*
FILING DATE: *March 23, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US04/33530*

Certified by

Jon W Dudas

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PTO/SB/16 (06-03)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV383801053US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Richard Augustin	Bond	Houston, Texas USA

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Methods for Treating Diseases and Conditions with Inverse Agonists and for Screening for Agents Acting as Inverse

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS ***(check all that apply)***

[X] Specification *Number of Pages* 38 [ ] CD(s), Number _____

[X] Drawing(s) *Number of Sheets* 2 [X] Other (specify) Abstract 1 sheets

[ ] Application Date Sheet. See 37 CFR 1.76

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[X] Applicant claims small entity status. See 37 CFR 1.27.

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[X] No.

[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____ Date March 23, 2004

TYPED or PRINTED NAME Michael B. Farber, Esq. REGISTRATION NO. 32,612 *(if appropriate)*

TELEPHONE (858) 450-0099 Docket Number: 8022-005-PR

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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FEE TRANSMITTAL for FY 2004

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☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	
Filing Date	March 23, 2004
First Named Inventor	Richard Augustin Bond
Examiner Name	
Art Unit	
Attorney Docket No.	8022-005-PR

METHOD OF PAYMENT *(check all that apply)*

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FEE CALCULATION

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1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X	=
Independent Claims		- 3** =		X	=
Multiple Dependent					=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

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FEE CALCULATION (continued)

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1051	130	2051	65	Surcharge - late filing fee or oath	
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1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

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SUBTOTAL (3) ($)

SUBMITTED BY

(Complete *if applicable*)

Name (Print/Type)	Michael B. Farber, Esq.	Registration No. (Attorney/Agent)	32,612	Telephone	858-450-0099
Signature	Michael B. Farber			Date	March 23, 2004

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March 23, 2004
Date of Deposit

March 23, 2004

Via Express Mail

Mail Stop Provisional Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Re: Provisional Patent Application
Methods for Treating Diseases and Conditions With Inverse Agonists and for Screening for Agents Acting as Inverse Agonists
Our File No. 8022-005-PR

Dear Commissioner:

Enclosed please find the following documents:

1. Provisional Application for Patent Cover Sheet (PTO/SB/16);
2. Fee Transmittal (PTO/SB/17)
3. Specification/Claims (39 pages)
4. Figures (2 pages)
5. Express Mail Certification Cover Letter (1 page)
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Please return the enclosed Post Card upon receipt and acceptance of this Petition. If you have any questions or need additional information, please do not hesitate to contact the undersigned directly.

Very truly yours,

Michael B. Farber, Esq.
Reg. No. 32,612

Enclosures

METHODS FOR TREATING DISEASES AND CONDITIONS WITH INVERSE AGONISTS AND FOR SCREENING FOR AGENTS ACTING AS INVERSE AGONISTS

Field of the Invention

This invention is directed to methods for treating diseases and conditions with inverse agonists, particularly with inverse agonists for G protein coupled receptors, and to methods for screening for agents capable of acting as inverse agonists, particularly as inverse agonists for G protein coupled receptors.

Background of the Invention

A large number of diseases and conditions are related to the activities of G protein coupled receptors (GPCR). The superfamily of G protein coupled receptors are integral membrane proteins characterized by amino acid sequences that contain seven hydrophobic domains, predicted to represent the transmembrane spanning regions of the proteins. They are found in a wide range of organisms and are involved in the transmission of signals to the interior of cells as a result of their interaction with heterotrimeric G proteins. They respond to a diverse range of agents including lipid analogues, amino acid derivatives, small molecules such as epinephrine and dopamine, and various sensory stimuli. The properties of many known GPCR are summarized in S.Watson & S. Arkinstall, "The G-Protein Linked Receptor Facts Book" (Academic Press, London, 1994), incorporated herein by this reference.

Among the diseases and conditions associated with the activity of GPCR are asthma and other obstructive respiratory diseases and congestive heart failure (CHF).

The incidence of asthma is increasing rapidly, particularly in children living in inner-city environments. The reasons for this increase are not clear, but various causes have been suggested, including dust mites, automobile-related pollution, and exposure to tobacco smoke. This disease is causing increasing morbidity and even mortality in many communities.

Patients with asthma and other airway disorders may have airway spasms, further reducing airflow through the pulmonary tree. During an attack, a patient's airway is constricted leading to difficulty breathing. Airway smooth muscle is responsible for the bronchoconstriction. The airway smooth muscle cells express β_2 adrenergic receptors. Agonist binding to these receptors, such as epinephrine or β_2 agonist drugs results in smooth muscle relaxation.

Consequently, for acute bronchospasms many patients inhale short-acting β_2 adrenergic agonists which function to immediately relax smooth muscle of the airway. Alternatively, asthmatics may take long-acting β_2 adrenergic agonists to prevent or reduce the severity of asthma attacks.

However, chronic administration of β-adrenergic agonists has been demonstrated to lead to drug tolerance. Furthermore, there is also an increased hyperresponsiveness of the pulmonary airway in response to provocation such as allergens.

Epidemiological studies have demonstrated a positive correlation between the chronic use of short-acting β-adrenergic agonists and asthma mortality. A large trial with the long-acting β_2-adrenergic agonist, salmeterol, was stopped due to increased death rates. This underscores that while short-term administration of β-agonists may be helpful to asthmatic patients, long-term administration may be deleterious.

Consequently, there is tremendous need for new therapeutic alternatives to β_2 agonist use in asthmatics. There is also a substantial need for new therapeutic alternatives for treating CHF and other diseases and conditions associated with GPCR.

Summary of the Invention

One aspect of the present invention is a method for treating a disease or condition associated with the activity of a G protein coupled receptor (GPCR) comprising administering an inverse agonist for the GPCR to an organism with a disease or condition associated with the activity of the GPCR in a quantity and for a period that causes an increase in the population of GPCRs, either spontaneously active or those that are available and activated by an endogenous agonist, associated with that physiological function, thereby producing a therapeutic effect to ameliorate the disease or condition. Another aspect of the invention is administering an inverse agonist for the GPCR to an organism with a disease or condition associated with the activity of the GPCR in a quantity and for a period that prevents the decrease in the population of GPCRs due to the presence of either exogenous or endogenous agonist.

Typically, the administration of the inverse agonist results in continuous levels of the inverse agonist in the bloodstream of the organism to which the inverse agonist is being administered.

The disease or condition can be a pulmonary airway disease, such as asthma, allergic rhinitis, bronchiectasis, bronchitis, chronic obstructive pulmonary disease (COPD), Churg-Strauss syndrome, cystic fibrosis, emphysema, or pneumonia.

When the disease or condition is a pulmonary airway disease, the therapeutic effect is typically a reduction in pulmonary airway constriction

hyperresponsiveness. When the disease or condition is a pulmonary airway disease, typically the GPCR is a β_2-adrenergic receptor, and the therapeutic effect is an upregulation of the population of these receptors. When the disease or condition is a pulmonary airway disease, the inverse agonist can be selected from the group consisting of nadolol, bupranolol, butoxamine, carazolol, carvedilol, ICI 118551, levobunolol, propranolol, sotalol, timolol, and the analogs or congeners of these drugs. Typically, the inverse agonist is nadolol or carvedilol.

The method can further comprise the administration of an additional agent, such as a β_2-selective adrenergic agonist drug, a steroid, an anticholinergic drug, an adenosine receptor antagonist, an anti-IgE antibody, a leukotriene modifier, or a phosphodiesterase-4 inhibitor.

Alternatively, the disease or condition can be congestive heart failure.

Alternatively, the disease or condition can be associated with the activity of a histamine H_1 receptor, such as chronic allergic rhinitis.

In still another alternative, the disease or condition can be associated with the activity of a histamine H_2 receptor, such as gastrointestinal reflux disease or stomach ulcers.

In yet another alternative, the disease or condition can be associated with acetylcholine receptors, α-adrenergic receptors, serotonin (5-hydroxytryptamine) receptors, dopamine receptors, adenosine receptors, angiotensin Type II receptors, bradykinin receptors, calcitonin receptors, calcitonin gene-related receptors, cannabinoid receptors, cholecystokinin receptors, chemokine receptors, cytokine receptors, gastrin receptors, endothelin receptors, γ-aminobutyric acid (GABA) receptors, galanin receptors, glucagon

receptors, glutamate receptors, luteinizing hormone receptors, choriogonadotrophin receptors, follicle-stimulating hormone receptors, thyroid-stimulating hormone receptors, gonadotrophin-releasing hormone receptors, leukotriene receptors, Neuropeptide Y receptors, opioid receptors, parathyroid hormone receptors, platelet activating factor receptors, prostanoid (prostaglandin) receptors, somatostatin receptors, thyrotropin-releasing hormone receptors, vasopressin and oxytocin receptors.

The method can further comprise the administration of an agonist to the GPCR along with the inverse agonist.

Another aspect of the invention is a method for screening a compound for inverse agonist activity against a GCPR comprising the steps of:

(1) providing a population of specific G protein coupled receptors characterized by a constitutive basal level of activity in the absence of an agonist;

(2) contacting the population of specific G protein coupled receptors with a compound to be screened for its inverse agonist activity, the compound not being an agonist of the population of specific G protein coupled receptors; and

(3) determining the constitutive basal level of activity of the specific G protein coupled receptors in the absence of the compound and in the presence of the compound, such that the constitutive basal level of activity decreases if the compound is an inverse agonist.

Yet another aspect of the invention is a method for screening a compound for inverse agonist activity against a GCPR comprising the steps of:

(1) providing cells containing a population of specific G protein coupled receptors characterized by a constitutive basal level of activity in the absence of an agonist;

(2) contacting the cells containing the population of specific G protein coupled receptors with a compound to be screened for its inverse agonist activity, the compound not being an agonist of the population of specific G protein coupled receptors, the compound being contacted with the cells for a period of time to result in an increase in receptor population or receptor density if the compound is an inverse agonist; and

(3) determining the receptor population or receptor density of the specific G protein coupled receptors in the cells in the absence of the compound and in the presence of the compound, such that the receptor population or receptor density increases if the compound is an inverse agonist.

Brief Description of the Drawings

These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description, appended claims, and accompanying drawings where:

Figure 1 is a series of graphs showing the effects of treatments with beta adrenergic drugs on airway responsiveness to methacholine in a murine model of asthma; and

Figure 2 is a series of photomicrographs showing immunohistochemical localization of beta-adrenergic receptors in the murine model, indicating increased receptor density after treatment with an inverse agonist.

Detailed Description of the Invention

The present invention provides for a general strategy based on previous unrecognized pharmacology of the effects of inverse agonists on G protein-coupled receptors. Compounds producing an acutely detrimental effect

via G protein-coupled receptors may provide a therapeutically beneficial effect with chronic administration and indicate that the chronic effect of the compounds cannot be predicted from their acute effects. Therefore, the present invention provides methods for treating diseases and conditions associated with the activity of G protein-coupled receptors. It further provides screening methods for detecting active agents that are inverse agonists and that are capable of treating diseases and conditions associated with the activity of G protein coupled receptors.

The basis of this strategy is the recognition of the existence of inverse agonists and the understanding of the effect that chronic treatment with inverse agonists has on receptor function. Receptors, such as β-adrenoceptors that respond to adrenalin (epinephrine), typically exist in an equilibrium between two states, an active state and an inactive state. When receptors bind to agonists, such as adrenalin for the β-adrenoceptors, they stop them from cycling back into the inactive state, thus shifting the equilibrium between the active and inactive states according to the Law of Mass Action. This occurs because those receptors bound to agonists are removed from the equilibrium. Typically, antagonists bind to the receptors, but prevent the binding of agonists. However, molecules known as "inverse agonists" bind to the receptors in the inactive state, causing the equilibrium between the active and the inactive states to shift toward the inactive state.

Moreover, there is a population of spontaneously active GPCRs *in vivo*. These receptors provide a baseline constitutive level of activity; the activity is never entirely "off."

Beta antagonists were also once contraindicated for congestive heart failure (CHF). However, extensive clinical trials have repudiated this and now the beta antagonist carvedilol is approved by the FDA as a first-line therapy

for CHF. Clinicians developed a very slow dosage ramping scheme to administer carvedilol safely to prevent any acute responses.

It is also well documented that chronic administration of beta adrenergic agonists cause agonist-dependent desensitization. Upon acute administration of beta agonists, adrenergic receptors are internalized thereby preventing them from being restimulated further for pulmonary relaxation. With chronic administration of beta agonists, there is actually a downregulation in the total number of beta adrenergic receptors. The consequence may be the observed loss of responsiveness seen in asthmatic patients on long-acting beta agonists.

The treatment methods of the present invention are based on the discovery that a chronic, low-dose administration of an inverse agonist has the effect of upregulating the population of spontaneously active GPCRs. This leads to the paradoxical result that the administration of a drug that would appear, at first blush, to degrade a physiological function, such as by causing airway hyperresponsiveness in asthma, can, if administered chronically, enhance that physiological function by upregulating the population of spontaneously active GPCRs associated with that physiological function.

Accordingly, in general, one aspect of the present invention is a method for treating a disease or condition associated with the activity of a G protein coupled receptor (GPCR) comprising administering an inverse agonist for the GPCR to an organism with a disease or condition associated with the activity of the GPCR in a quantity and for a period that causes an increase in the population of GPCRs, either spontaneously active or those that are available and activated by an endogenous agonist, associated with that physiological function, thereby producing a therapeutic effect to ameliorate the disease or condition. Another aspect of the invention is administering an inverse agonist for the GPCR to an organism with a disease or condition associated with the activity of the

GPCR in a quantity and for a period that prevents the decrease in the population of GPCRs due to the presence of either exogenous or endogenous agonist.

Typically, the chronic administration of an inverse agonist provides a therapeutic benefit equivalent or greater than the therapeutic effect of the administration of an acute agonist.

Typically, the method of administration of the inverse agonist results in continuous levels of the inverse agonist in the bloodstream of the organism to which the inverse agonist is being administered.

The disease or condition associated with the activity of the GPCR can be a pulmonary airway disease. Typically, the pulmonary airway disease is asthma. Alternatively, the pulmonary airway disease is allergic rhinitis, bronchiectasis, bronchitis, chronic obstructive pulmonary disease (COPD), Churg-Strauss syndrome, cystic fibrosis, emphysema, or pneumonia.

When the disease or condition associated with the activity of a GPCR is a pulmonary airway disease, the therapeutic effect can be a reduction in pulmonary airway constriction hyperresponsiveness.

When the disease or condition associated with the activity of a GPCR is a pulmonary airway disease, the GPCRs can be β_2-adrenergic receptors. The therapeutic effect can be an upregulation of the population of pulmonary β_2-adrenergic receptors. The therapeutic effect can also be increased pulmonary airway relaxation responsiveness to β_2-adrenergic agonist drugs.

When the inverse agonist administered is an inverse agonist for a β_2-adrenergic receptor, the inverse agonist can be selected from the group consisting of nadolol, bupranolol, butoxamine, carazolol, carvedilol, ICI 118551, levobunolol, propranolol, sotalol, timolol, and the analogs or congeners of these

drugs. Typically, the inverse agonist for the β_2-adrenergic receptor is nadolol or carvedilol.

In addition, prodrugs and salt forms of these compounds are encompassed by the present invention. It is well known that organic compounds, including compounds having activities suitable for methods according to the present invention, have multiple groups that can accept or donate protons, depending upon the pH of the solution in which they are present. These groups include carboxyl groups, hydroxyl groups, amino groups, sulfonic acid groups, and other groups known to be involved in acid-base reactions. The recitation of a compound or analogue includes such salt forms as occur at physiological pH or at the pH of a pharmaceutical composition unless specifically excluded.

Similarly, prodrug esters can be formed by reaction of either a carboxyl or a hydroxyl group on compounds or analogues suitable for methods according to the present invention with either an acid or an alcohol to form an ester. Typically, the acid or alcohol includes a lower alkyl group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tertiary butyl. These groups can be substituted with substituents such as hydroxy, or other substituents. Such prodrugs are well known in the art and need not be described further here. The prodrug is converted into the active compound by hydrolysis of the ester linkage, typically by intracellular enzymes. Other suitable groups that can be used to form prodrug esters are well known in the art.

In addition, where compounds recited above are optically active, both the optically active form and the racemic mixture are encompassed by the present invention unless the racemic mixture is specifically excluded.

The compounds also can be prepared as pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include acid addition salts such as those containing hydrochloride, sulfate, phosphate,

sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, cyclohexylsulfamate and quinate. (See e.g., PCT Patent Application No. PCT/US92/03736, incorporated herein by this reference). Such salts can be derived using acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, and quinic acid.

Pharmaceutically acceptable salts can be prepared by standard techniques. For example, the free base form of the compound is first dissolved in a suitable solvent such as an aqueous or aqueous-alcohol solution, containing the appropriate acid. The salt is then isolated by evaporating the solution. In another example, the salt is prepared by reacting the free base and acid in an organic solvent.

Carriers or excipients can be used to facilitate administration of the compound, for example, to increase the solubility of the compound. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See, e.g. A.S. Nies & S.P. Spielberg, "Principles of Therapeutics" in J.G. Hardman & L.E. Limbird, eds., "Goodman & Gilman's The Pharmacological Basis of Therapeutics" (9th ed., McGraw-Hill, New York, 1996), ch. 3., pp. 43-62. It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The

magnitude of an administered dose in the management of a disease or condition associated with the activity of a GPCR will vary with the severity of the disease or condtion and with the route of administration. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient, as well as other conditions affecting pharmacodynamic parameters such as liver and kidney function. However, in general, treatment will begin with a low dose, typically a dose considered subclinical in respect to the generally accepted use of the inverse agonist, and the dosage will be increased over time.

When the disease or condition associated with the activity of a GPCR is a pulmonary airway disease, the method for treating the disease or condition can further comprise the administration of an additional agent. In one alternative, the additional agent is a β_2-selective adrenergic agonist drug. The β_2-selective adrenergic agonist drug can be albuterol, bitolterol, dobutamine, fenoterol, formoterol, levalbuterol, pirbuterol, salbutamol, salmeterol, or terbutaline.

In another alternative, the additional agent is a steroid. The steroid can be beclomethasone, budesonide, ciclesonide, flunisolide, fluticasone, methylpredisolone, prednisolone, prednisone, or triamcinolone.

In yet another alternative, the additional agent is an anticholinergic drug. The anticholinergic drug can be ipratropium or tiotropium.

In yet another alternative, the additional agent is an adenosine receptor antagonist. The adenosine receptor antagonist can be theophylline, theobromine or caffeine.

In yet another alternative, the additional agent is an anti-IgE antibody. The anti-IgE antibody can be omalizumab.

In yet another alternative, the additional agent is a leukotriene modifier. The leukotriene modifier can be ibudilast, montelukast, pranlukast, or zafirlukast.

In yet another alternative, the additional agent is a phosphodiesterase-4 inhibitor. The phosphodiesterase-4 inhibitor can be roflumilast or cilomilast.

In another alternative, the disease or condition associated with the activity of a GPCR can be congestive heart failure (CHF). When the disease or condition associated with the activity of a GPCR is CHF, the GPCRs are also β_2-adrenergic receptors, and the inverse agonist is typically carvedilol or nadolol.

In another alternative, the disease or condition associated with the activity of a GPCR can be a disease or condition associated with the activity of the histamine H_1 receptor. In this alternative, the disease or condition can be chronic allergic rhinitis (N. Iriyoshi et al., "Increased Expression of Histamine H1 Receptor mRNA in Allergic Rhinitis," Clin. Exp. Allergy 26: 379-385 (1996)) or another disease or condition for which a histamine H_1 receptor antagonist is commonly administered. In this alternative, the inverse agonist is typically administered together with a H_1 agonist such as histamine itself or a histamine analogue. Currently used histamine H_1 receptor antagonists have a number of well-recognized side effects, such as sedation, loss of appetite, nausea, vomiting, epigastic distress, and constipation or diarrhea. In rare cases, currently used histamine H_1 receptor antagonists can cause polymorphic ventricular tachycardia. Other side effects are known.

In yet another alternative, the disease or condition associated with the activity of a GPCR can be a disease or condition associated with the activity of the histamine H_2 receptor. In this alternative, the disease or condition can be

gastrointestinal reflux disease (GERD), stomach ulcers, or another disease or condition for which a histamine H_2 receptor antagonist is commonly administered.

The combination of a histamine H_2 receptor inverse agonist with an histamine H_2 agonist can be used for the treatment of gastrointestinal acid reflux disease without the development of tolerance or rebound intragastric hyperacidity upon discontinuation of treatment. High levels of a histamine H_2 inverse agonist function to inhibit the activity of the histamine H_2 receptor and low levels of a histamine receptor agonist function to prevent receptor up-regulation, preventing the development of tolerance to the inverse agonist and preventing rebound hyperacidity.

Problems with current histamine H_2 blocking drugs include the fact that chronic administration of H_2 blockers leads to tolerance and loss of efficacy of the drug (C.U. Nwokolo et al., "Tolerance During 5 Months of Dosing with Ranitidine, 150 mg Nightly: a Placebo-Controlled, Double-Blind Study," Gastroenterology 101: 948-953 (1991) C.H. Wilder-Smith et al., "Tolerance to Oral H_2-Receptor Antagonists," Dig. Dis. Sci. 35: 976-983 (1990); C.U. Nwokolo et al., "Tolerance During 29 Days of Conventional Dosing with Cimetidine, Nizatidine, Famotidine or Ranitidine," Aliment. Pharmacol. Ther. 4 (Suppl. 1) 29-45 (1990)). There is also rebound gastric hyperacidity upon cessation of H_2 blocker treatment (C.U. Nwokolo et al., "Rebound Intragastric Hyperacidity After Abrupt Withdrawal of Histamine H_2 receptor Blockade," Gut 12: 1455-1460 (1991)).

Side effects possibly due to H_2 inverse agonists include possible stimulation or up-regulation of spontaneously active histamine H_2 receptors (M.J. Smit et al., "Inverse Agonism of Histamine H2 Antagonists Accounts for Upregulation of Spontaneously Active Histamine H2 Receptors," Proc. Natl. Acad. Sci. USA 93: 6802-6807 (1996)). However, the effects of these can be

controlled by appropriate use of the agonist in combination therapy as described above.

H_2 inverse agonists include cimetidine, nizatidine, ranitidine, and famotidine.

H_2 agonists include histamine itself (agonist at all histamine receptors), dimaprit, betazole, ametazole, and arpromidine. Burimamide functions as a H_2 neutral antagonist.

Methods according to the present invention can also be used for the treatment of diseases and conditions associated with the activity of other GPCRs, including, but not limited to, acetylcholine receptors (including muscarinic receptors), α-adrenergic receptors, serotonin (5-hydroxytryptamine) receptors, dopamine receptors, adenosine receptors, angiotensin Type II receptors, bradykinin receptors, calcitonin receptors, calcitonin gene-related receptors, cannabinoid receptors, cholecystokinin receptors, chemokine receptors, cytokine receptors, gastrin receptors, endothelin receptors, γ-aminobutyric acid (GABA) receptors, galanin receptors, glucagon receptors, glutamate receptors, luteinizing hormone receptors, choriogonadotrophin receptors, follicle-stimulating hormone receptors, thyroid-stimulating hormone receptors, gonadotrophin-releasing hormone receptors, leukotriene receptors, Neuropeptide Y receptors, opioid receptors (Lesscher et al., Eur. J. Neurosci. 17: 1006-1012 (2003)) , parathyroid hormone receptors, platelet activating factor receptors, prostanoid (prostaglandin) receptors, somatostatin receptors, thyrotropin-releasing hormone receptors, vasopressin and oxytocin receptors, and other physiologically active receptors.

In particular, diseases and conditions associated with the activity of the opioid receptors are significant. The use of inverse agonists together with agonists to these receptors can allow pain management without the tolerance

associated with opioid-mediated down-regulation of opioid receptors and the ability of opioid antagonists to prevent opioid receptor internalization (S.M. Crain & K.F. Shen, "Ultra-Low Concentrations of Naloxone Selectively Antagonize Excitatory Effects of Morphine on Sensory Neurons, Thereby Increasing Its Antinociceptive Potency and Attenuating Tolerance/Dependence During Chronic Cotreatment," Proc. Natl. Acad. Sci. 92: 10540-10544 (1995); A. Tempel et al., "Morphine-Induced Downregulation of μ-Opioid Receptors in Neonatal Rat Brain," Brain Res. 469: 129-133 (1988); N. Marie et al., "Differential Sorting of Human δ-Opioid Receptors After Internalization by Peptide and Alkaloid Antagonists," J. Biol. Chem. 278: 22795-22804 (2003); C.N. Patel et al., "Chronic Opioid Antagonist Treatment Selectively Regulates Trafficking and Signaling Proteins in Mouse Spinal Cord," Synapse 50: 67-76 (2003)). This can provide improved pain management.

These methods can also further comprise the administration of an appropriate agonist to the GPCR.

Methods according to the present invention can further be used for the treatment of diseases and conditions associated with GPCRs disclosed in G. Milligan & R.A. Bond, "Inverse Agonism and the Regulation of Receptor Number," Trends Pharmacol. Sci. 12: 468-474 (1997), incorporated herein by this reference, and in R.A. Bond, "Is Paradoxical Pharmacology a Strategy Worth Pursuing?," Trends Pharmacol. Sci. 22: 273-276 (2001), also incorporated herein by this reference.

Methods according to the present invention can be used in human patients. Alternatively, methods according to the present invention can be used in socially or economically important animals such as dogs, cats, cattle, sheep, pigs, goats, and horses.

Another aspect of the present invention is a screening method for detecting active agents that are inverse agonists and that are capable of treating diseases and conditions associated with the activity of G protein coupled receptors. In general, such a screening method comprises:

(1) providing a population of specific G protein coupled receptors characterized by a constitutive basal level of activity in the absence of an agonist;

(2) contacting the population of specific G protein coupled receptors with a compound to be screened for its inverse agonist activity, the compound not being an agonist of the population of specific G protein coupled receptors; and

(3) determining the constitutive basal level of activity of the specific G protein coupled receptors in the absence of the compound and in the presence of the compound, such that the constitutive basal level of activity decreases if the compound is an inverse agonist.

The constitutive basal level of activity of the specific G protein coupled receptors in the absence and in the presence of the compound can be measured by various techniques, depending on whether intact organisms, cell cultures, or tissue cultures are being used. For example, the production or activity of a second messenger such as cyclic AMP (cAMP) can be measured. If intact organisms are used, the physiological consequences of receptor activation, such as airway resistance, can be measured. In many systems, it is desirable to transform or transfect cells with genetically engineered constitutively active mutant receptors (CAM). This can be done by standard genetic engineering techniques. Alternatively, overexpression of the wild-type receptors can be induced. These approaches are described in R.A.F. de Ligt et al., "Inverse Agonism at G Protein-Coupled Receptors: (Patho)physiological Relevance and Implications for Drug Discovery," Br. J. Pharmacol. 130: 1-12 (2000), incorporated herein by this reference.

This screening method can be used to detect active agents that are inverse agonists for β_2-adrenergic receptors, H_1 receptors, H_2 receptors, and other receptors described above. Therefore, this screening method can be used to detect agents that can be used to treat diseases and conditions associated with these receptors, including, but not limited to, pulmonary airway diseases, including asthma, chronic allergic rhinitis, gastrointestinal reflux disease, and stomach ulcers.

Another screening method according to the present invention relies on the finding, described above, that exposure of cells containing a specific population of G protein coupled receptors to an inverse agonist for a substantial period of time results in an increase in receptor population or receptor density in the cells. Therefore, this alternative of the screening method comprises:

(1) providing cells containing a population of specific G protein coupled receptors characterized by a constitutive basal level of activity in the absence of an agonist;

(2) contacting the cells containing the population of specific G protein coupled receptors with a compound to be screened for its inverse agonist activity, the compound not being an agonist of the population of specific G protein coupled receptors, the compound being contacted with the cells for a period of time to result in an increase in receptor population or receptor density if the compound is an inverse agonist; and

(3) determining the receptor population or receptor density of the specific G protein coupled receptors in the cells in the absence of the compound and in the presence of the compound, such that the receptor population or receptor density increases if the compound is an inverse agonist.

The receptor population or receptor density can be determined by an immunochemical method, using labeled antibodies specific for the receptor,

although other methods can be used. The use of such labeled antibodies is well known in the art and need not be described further here; radioactive labels or fluorescent labels can be used. This is used in Example 3, below. Alternatively, the receptor population or receptor density can be determined by the binding of a radioligand with an affinity sufficiently high to bind all receptors and measuring the extent of binding. This is used in Example 2, below.

This screening method can also used to detect active agents that are inverse agonists for β_2-adrenergic receptors, H_1 receptors, H_2 receptors, and other receptors described above. Therefore, this screening method can also be used to detect agents that can be used to treat diseases and conditions associated with these receptors, including, but not limited to, pulmonary airway diseases, including asthma, chronic allergic rhinitis, gastrointestinal reflux disease, and stomach ulcers.

These treatment and screening methods are significant. GPCRs account for 60% of drug targets on market, e.g. beta agonists, alpha blockers, beta blockers, H1 & H2 blockers, and other targets. Drug hiscovery for GPCR targets has generally been focused on "acute" effects at protein, cellular level and in animals. Physicians and scientists have generally extrapolated that chronic effects of drug would be identical to acute drug effects. However, the present invention has shown that the acute effects of many drugs do not equal their chronic effects, and that this can be exploited to identify new drug therapeutics. This is a general, previously unrecognized phenomenon that explains paradoxical benefit effects of drugs in various therapeutic indications. The present invention demonstrates this for beta inverse agonist use in asthma and explains the efficacy of this drug class in CHF as not being an isolated example as most in the field view it.

For CHF the acute effects of beta agonists do not equal their chronic effects; beta agonists helpful acutely but increase mortality chronically.

The serendipitous discovery that beta blockers have huge benefit in reducing mortality upon chronic administration despite short-term acute detriment has been generally viewed as a single isolated incidence of inherent paradox. However, the present invention makes clear that a novel route of drug action and a novel method of drug discovery underlies this seemingly isolated finding.
The present invention makes clear that that there are many examples of GPCRs that are up-regulated by inverse agonists. These observations have not been completely valued till now. The present invention also demonstrates that GPCRs (for example, β_2-adrenoceptors in transgenic mouse) are spontaneously active in absence of agonist.

Whilst not being held to this theory, the inventor believes that part of the explanation for the therapeutic effect of chronic administration of inverse agonists is the up-regulation of spontaneously active GPCRs, this may also include upregulation of internal components of signal transduction pathway.

The present invention also incorporates the finding, first *in vitro* then *in vivo*, that GPCRs have activity in the absence of ligand. This new appreciation of GPCRs impacts our understanding that there are three classes of drugs that can interact with two different forms of a GPCR.

The invention is illustrated by the following Examples. These Examples are for illustrative purposes only and are not intended to limit the invention.

Example 1

Airway Resistance Reduction by Chronic Administration of Beta Adrenergic Inverse Agonists

Methods

Balb/cJ mice aged 6 weeks (Jackson Animal Laboratory, Bar Harbor, Maine) were housed under specific pathogen-free conditions and fed a chicken ovalbumin-free diet. The Animal Research Ethics Boards of both the University of Houston and Baylor College of Medicine approved all experiments reported here. The effects of administration of the non-selective beta antagonists carvedilol (GlaxoSmithKline, King of Prussia, PN), nadolol (Sigma Chemical, St. Louis, MO), and of salbutamol (Sigma Chemical, St. Louis, MO), a β_2 adrenergic partial agonist, were examined in a murine model that exhibited cardinal features of human asthma, such as pulmonary eosinophilic inflammation, airway hyperresponsiveness, and heterogenous airway narrowing. The results obtained in drug-treated animals were compared with those obtained in vehicle-treated counterparts in experiments performed in close temporal relationship. The outcome measures of this study included statistically-significant differences between drug-treated mice and non-treated animals in terms of baseline airway resistance, degree of airway responsiveness to cholinergic stimulation, and bronchoalveolar lavage (BALF) cellularity. Mice were sensitized with subcutaneous injection of 25 μg of ovalbumin adsorbed to aluminum hydroxide on protocol days 2, 9, and 16. Subsequently, mice were given 50 μl of saline solution containing 25 μg of ovalbumin intranasally, on a daily basis, from protocol days 23 through 27. A group of ovalbumin-sensitized saline-challenged mice served as controls for systemic sensitization and respiratory challenges with ovalbumin. Prior to intranasal administrations, mice were sedated with halothane vapor. For this study, ovalbumin-sensitized and ovalbumin-challenged mice, and ovalbumin-sensitized and saline-challenged mice will be referred to as asthmatic mice and control mice, respectively. The drugs used were salbutamol (a β_2 adrenergic partial agonist), aprenolol (a β_1/β_2 adrenergic antagonist with partial β_2 agonist activity), nadolol and carvedilol (both nonselective β_1/β_2 adrenergic antagonists with inverse agonist activity at the β_2 adrenergic receptor).

To examine the effects of duration of beta adrenergic ligand therapy on the phenotype of the murine model of asthma, the experimental drugs were administered either acutely or chronically to different groups of asthmatic mice.

Asthmatic mice on acute therapy were given a single intravenous bolus infusion of either beta adrenergic drug or normal saline on protocol day 28, 15 minutes before airway responsiveness to methacholine was determined. The doses of carvedilol, nadolol, aprenolol, and salbutamol administered to the mice were 24 mg/kg, 72 mg/kg, 72 mg/kg, and 0.15 mg/kg, respectively. Asthmatic mice on chronic therapy were treated with the beta adrenergic drug during protocol days 1 to 28. Those on beta antagonists had free access to chow treated with carvedilol, nadolol, or alprenolol at concentrations of 2400 ppm, 250 ppm, or 7200 ppm, respectively. These concentrations were chosen based on those producing therapeutic effects in mice in previously published studies. The non-treated asthmatic mice were fed normal chow. Salbutamol was delivered for 28 days at a dose of 0.5 mg/kg/day using an osmotic minipump (Alzet®, #2004, Durect Corporation, Cupertino, CA).

On protocol day 28, mice were anesthetized, tracheotomized, and connected to a computer-controlled small animal ventilator (Flexivent, Scientific Respiratory Equipment, Inc., Montreal, Canada). Airway resistance (R_{aw}) was measured using the forced oscillation technique. The cellular composition of bronchoalveolar lavage fluid (BALF) was also determined. In non-treated asthmatic mice, the degree of airway responsiveness and the number of eosinophils recovered in BALF were significantly higher compared to the ovalbumin-sensitized saline-challenged (control) mice. However, it was observed that the degree of airway responsiveness and the number of eosinophils recovered in BALF were lower in non-treated asthmatic mice studied in close temporal relationship with mice receiving acute beta adrenergic antagonist

treatments than in those obtained in non-treated asthmatic mice studied concomitantly with mice on chronic beta adrenergic antagonist therapy.

To induce airway constriction, a solution containing 150 μg/ml of acetyl-α-methylcholine chloride (methacholine) (Sigma Chemical, St. Louis, MO) was infused intravenously at constant rates using a syringe infusion pump (Raze Scientific Instruments, Stanford, CN). The methacholine infusion was started at 0.008 ml/min, and its rate was doubled stepwise up to a maximum of 0.136 ml/min. Each methacholine dose was administered for 3 to 5 minutes, during which data were sampled at 1 minute intervals and then averaged.

Data Analysis

The complex input impedance of the respiratory system was computed and the value of the real part of respiratory system impedance at 19.75 Hz was taken to reflect the magnitude of airway resistance (R_{aw}). To examine the degree of airway responsiveness of each animal, the values for R_{aw} as a function of methacholine doses were plotted. The largest value for R_{aw} obtained in response to methacholine stimulation was referred to as R_{awpeak}. For mice that achieved a plateau in the methacholine dose-R_{aw} response curve, the ED_{50} was calculated by linear interpolation using the GraphPad Prism4 (GraphPad Software, Inc.). Results were expressed as mean±SEM. Comparisons between results obtained for beta adrenergic drug treated and non-treated mice were performed using the analysis of variance for multiple groups of a student's t-test for comparing two groups. The Bonferroni test was used to examine the statistical differences between experimental groups. The effects of acute drug treatments on baseline respiratory system mechanics were assessed using two-tailed paired t-test. A value of $P<0.05$ was considered statistically significant.

Figure 1 shows the effects of treatments with beta adrenergic drugs on airway responsiveness to methacholine in a murine model of asthma. Asthmatic mice received either a single intravenous bolus injection 15 minutes prior to methacholine challenge (acute; top row) or were treated for 28 days (chronic; bottom row). Average methacholine dose-airway resistance relationships were obtained in control mice (Ctrl o, N = 6-21), non-treated asthmatic mice (NTX •, N = 7-25), and in asthmatic mice treated with the beta adrenergic drugs (□, N = 8-19). Values are mean ±SEM. Please note the change in the scale of the y-axis for panels G and I

Panels A and B: no drug treatment, control mice and non-treated asthmatic mice.

Panels C and D: salbutamol treatment.

Panels E and F: alprenolol treatment.

Panels G and H: carvedilol treatment.

Panels I and J: nadolol treatment.

Figures 1A and 1B show that methacholine provocation significantly enhances airway resistance (R_{aw}) in asthmatic mice in contrast to a minimal response upon saline provocation of asthmatic mice. This demonstrates that the mouse model in this study exhibits airway hyperresponsiveness, a key feature of airway dysfunction in human asthma.

In Figure 1C, the administration of a single intravenous bolus of salbutamol to asthmatic mice reduced the level of airway responsiveness to methacholine provocation and the level of airway resistance as expected. In Figure 1D when salbutamol was delivered for 28 days to the mice, no protection

was observed. This lack of reduction of airway hyperresponsiveness upon chronic administration of a beta adrenergic agonist has been observed in humans when tolerance to these drugs develop.

In Figure 1E, when asthmatic mice were given a single intravenous bolus of alprenolol, a beta adrenergic antagonist with partial agonist activity, their airway responsiveness was diminished, as indicated by significant decreases in both the values for R_{aw} at methacholine doses ≥408 μg/kg/min. ($P<0.05$) compared to those obtained in non-treated counterparts. The reduction in airway responsiveness upon acute administration of alprenolol is similar to that observed for salbutamol, consistent with the partial agonist activity that alprenolol possesses. In Figure 1F, when asthmatic mice were exposed to alprenolol for 28 days, their average methacholine dose-response relationship was similar to that obtained in nontreated mice demonstrating that provides no benefit upon chronic administration as is the case with salbutamol.

In Figure 1G, a single intravenous bolus of carvedilol enhanced the airway responsiveness in the asthmatic mice. This is consistent to previous observations in humans that acute delivery of beta adrenergic antagonists to asthmatics can result in severe airway constriction. In contrast, in Figure 1H, chronic administration of carvedilol reduced the responsiveness of asthmatic mice to methacholine provocation. Chronic delivery of carvedilol not only decreased the airway constrictor response at high doses of methacholine, it also shifted the methacholine dose-airway response relationship to the left of that obtained in the non-treated asthmatic mice.

In Figure 1I, a single intravenous bolus of nadolol also enhanced the airway responsiveness of asthmatic mice similar to that observed for carvedilol. Chronic delivery of nadolol shown in Figure 1J also produced a decrease in airway responsiveness, which was more pronounced that that caused by long-term carvedilol treatment. Indeed, the average methacholine

dose-R_{aw} response relationship obtained in asthmatic mice on chronic nadolol treatment was similar to that obtained in mice on acute salbutamol treatment.

Example 2

Chronic Inverse Agonist Treatment Increases Beta Adrenergic Receptor Numbers as Measured by Radioligand Binding

Asthmatic mice (ovalbumin-sensitized) were treated as follows: Ctrl, no drug treatment and no methacholine challenge; NTX, no drug treatment with methacholine challenge; salbutamol, a short-acting β_2 agonist, carvedilol, a β_1, β_2 non-selective inverse agonist with α_1 adrenergic antagonist activity, and nadolol, a highly specific, hydrophillic β_1/β_2 non-selective adrenergic inverse agonist. Drug treatments were either a single treatment 15 minutes prior to methacholine challenge or ongoing for 28 days (salbutamol was delivered continuously via a subcutaneous osmotic minipump and alprenolol, carvedilol, and nadolol were in animal chow).

β_2 adrenergic receptor numbers were measured in non-drug-treated asthmatic mice and in asthmatic mice chronically-treated with the beta adrenergic inverse agonist, carvedilol and the beta adrenergic antagonist, alprenolol. Mice were sacrificed and lung membranes were isolated as follows. Frozen lung tissue was homogenized in an ice-cold buffer containing 0.32 M sucrose and 25 mM Tris (pH 7.4) using a polytron (Pro 200, Pro Scientific, Inc.). The homogenate was centrifuged at 1000 *g* for 10 min at 4°C. This supernatant was centrifuged at 40,000 *g* for 20 min at 4°C. The pellet was suspended in an ice-cold 25 mM Tris-HCl buffer (pH 7.4) and centrifuged at 40000 *g* for 20 min at 4°C. The final pellet was suspended in 200 µl 25 mM Tris-HCl (pH 7.4), membrane protein concentration was determined by BCA protein assay kit. Radioligand receptor binding incubation mixtures contain membranes (~10 µg of

protein), (-)3-[125I]-cyanopindolol (ICYP) in 25mM Tris-HCl, pH 7.4) in increasing concentrations (5–7500 pM) and binding buffer in a final volume of 250 µl. Propranolol was used to determine nonspecific binding. The incubation was done at 37°C for 2 h and terminated by rapid vacuum filtration through glass fiber filters. The filters were washed three times with 250 µl of ice cold wash buffer (25 mM Tris-HCl, pH 7.4) and the radioactivity determined in a counter. All experiments were performed in triplicate and receptor densities are expressed as picomoles of sites per milligram of protein. Bmax is determined by nonlinear regression of the saturation binding curves. ß-AR density was measured in membranes prepared from lung tissue using the ß-AR radioligand ICYP in increasing concentrations (5-7,500 pM). Samples were run in triplicate and values are mean ± s.e.m. of n=3-5 animals in each group. Bmax are expressed in fM mg^{-1} and apparent KD values (in parenthesis) are expressed as pM. Please note, the 15 minute and 28 day time point refers to duration of drug treatment. All mice were killed at the same age and thus for vehicle treated groups (Ctrl and NTX) the groups were identical and the results pooled. #P<0.05 compared to Ctrl; *P<0.05 compared to NTX (Student's t-test) (ANOVA, Bonferoni correction) (Table 1).

Radioligand binding revealed that β_2 adrenergic receptor levels are not altered merely by the absence or presence of methacholine challenge as seen by the essentially similar levels of β_2 adrenergic receptors in both the methacholine-challenged and the unchallenged non-drug treated asthmatic mice as shown in Table 1. Chronic alprenolol treatment led to a slight doubling of the level of the β_2 adrenergic receptor. Most significantly, was the over 10-fold increase of β_2 adrenergic receptors in the carvedilol-treated mice over the non-treated mice, demonstrating the efficacy of this beta adrenergic inverse agonist in increasing receptor levels upon chronic administration.

Table 1. Determination of β-AR density by radioligand binding.

Treatment	15 minutes		28 days	
	Bmax (fmol mg^{-1} protein)	K_D (pM)	Bmax (fmol mg^{-1} protein)	K_D (pM)
Ctrl	286.8 ± 88.02	(107.9 ± 43.67)	286.8 ± 88.02	(107.9 ± 43.67)
NTX	109.2 ± 9.72 #	(193.6 ± 20.66)	109.2 ± 9.72 #	(193.6 ± 20.66)
Salbutamol	256.5 ± 29.24 *	(228.8 ± 33.07)	97.0 ± 23.02	(225.4 ± 41.79)
Alprenolol	299.5 ± 12.19 *	(453.6 ± 86.33)	179.2 ± 53.05	(290.9 ± 55.07)
Carvedilol	86.3 ± 19.42	(565.2 ± 192.8) *	904.1 ± 43.46 *	(1444.0 ± 202.0) *
Nadolol	181.9 ± 48.28	(695.1 ± 286.3) *	785.5 ± 164.8 *	(1591.6 ± 335.0) *

Example 3

Chronic Inverse Agonist Treatment Increases Beta Adrenergic Receptor Numbers as Monitored by Immunohistochemistry

For immunohistochemistry analysis of β_2 adrenergic receptor levels, non-drug-treated control mice and mice treated chronically with the beta adrenergic inverse agonist nadolol were used. The mice were sacrificed and the lungs excised. Then the lungs were fixed in 4% paraformaldehyde (45 min, 0°C). After fixation, lungs were washed in PBS (60 min) and placed in increasing concentrations of sucrose (10% sucrose/5% glycine in PBS for 30 min; 20% sucrose/10% glycine in PBS for 30 min; 30% sucrose/15% glycine in PBS for 12 h at 4°C). Lungs were embedded in OCT and 12 µm sections cut with a Tissue-Tek II cryostat. The sections were air dried and fixed with 4% paraformaldehyde for 15 min. After 3 washes in PBS, the slides were blocked with 5% milk in PBS for 1 h, and then incubated overnight with anti-β_2 adrenergic receptor antibody (1:200; Santa Cruz Biotechnology) in blocking solution. Slides were washed in PBS and incubated with secondary antibody (1:200; Cy3-goat anti-rabbit, 16 h at 4°C). Control slides were incubated with antibody specific blocking peptide to demonstrate specificity of binding of the primary antibody. After washing with PBS, coverslips were mounted and viewed by epifluorescent microscopy.

For the results shown in Figure 2, lung sections from non-drug-treated mice and from chronically-treated nadolol mice were stained with anti-β_2 adrenergic receptor antibodies in the presence and absence of competing β_2 adrenergic receptor peptide. In panel A, very little staining is present in the non-drug-treated mice whereas in panel C, the nadolol-treated mice had a significant level of staining. In panels B and D, addition of the competing peptide eliminated all signals demonstrating that the original signals were due to the presence of β_2 adrenergic receptors.

As shown in Figure 2, labeling with anti-β_2 adrenergic receptor antibodies was considerably more intense in lungs from treated animals than in lungs from animals not treated with nadolol. Loss of this signaling upon incubation in the presence of the β_2 adrenergic receptor peptide, demonstrates that this antibody is specifically binding the β_2 adrenergic receptor. This observation is consistent with the radioligand binding data and suggests β_2 adrenergic receptors are effectively upregulated by chronic administration of beta adrenergic inverse agonist drugs.

I claim:

1. A method for treating a disease or condition associated with the activity of a G protein coupled receptor (GPCR) comprising administering an inverse agonist for the GPCR to an organism with a disease or condition associated with the activity of the GPCR in a quantity and for a period that causes an increase in the population of GPCRs, either spontaneously active or those that are available and activated by an endogenous agonist, associated with that physiological function, thereby producing a therapeutic effect to ameliorate the disease or condition.

2. The method of claim 1 wherein the administration of the inverse agonist results in continuous levels of the inverse agonist in the bloodstream of the organism to which the inverse agonist is being administered.

3. The method of claim 1 wherein the disease or condition associated with the activity of a GPCR is a pulmonary airway disease.

4. The method of claim 3 wherein the pulmonary airway disease is asthma.

5. The method of claim 3 wherein the pulmonary airway disease is selected from the group consisting of allergic rhinitis, bronchiectasis, bronchitis, chronic obstructive pulmonary disease (COPD), Churg-Strauss syndrome, cystic fibrosis, emphysema, and pneumonia.

6. The method of claim 3 wherein the therapeutic effect is a reduction in pulmonary airway constriction hyperresponsiveness.

7. The method of claim 3 wherein the GPCR is a β_2-adrenergic receptor.

8. The method of claim 7 wherein the therapeutic effect is an upregulation of the population of pulmonary β_2-adrenergic receptors.

9. The method of claim 7 wherein the therapeutic effect is increased pulmonary airway relaxation responsiveness to β_2-adrenergic agonist drugs.

10. The method of claim 7 wherein the inverse agonist is selected from the group consisting of nadolol, bupranolol, butoxamine, carazolol, carvedilol, ICI 118551, levobunolol, propranolol, sotalol, timolol, and the analogs or congeners of these drugs.

11. The method of claim 10 wherein the inverse agonist is nadolol or carvedilol.

12. The method of claim 3 wherein the method further comprises the administration of an additional agent.

13. The method of claim 12 wherein the additional agent is β_2-selective adrenergic agonist drug.

14. The method of claim 13 wherein the β_2-selective adrenergic agonist drug is selected from the group consisting of albuterol, bitolterol, dobutamine, fenoterol, formoterol, levalbuterol, pirbuterol, salbutamol, salmeterol, and terbutaline.

15. The method of claim 12 wherein the additional agent is a steroid.

16. The method of claim 15 wherein the steroid is selected from the group consisting of beclomethasone, budesonide, ciclesonide, flunisolide, fluticasone, methylpredisolone, prednisolone, prednisone, and triamcinolone.

17. The method of claim 12 wherein additional agent is an anticholinergic drug.

18. The method of claim 17 wherein the anticholinergic drug is selected from the group consisting of ipratropium and tiotropium.

19. The method of claim 12 wherein the additional agent is an adenosine receptor antagonist.

20. The method of claim 19 wherein the adenosine receptor antagonist is selected from the group consisting of theophylline, theobromine, and caffeine.

21. The method of claim 12 wherein the additional agent is an anti-IgE antibody.

22. The method of claim 21 wherein the anti-IgE antibody is omalizumab.

23. The method of claim 12 wherein the additional agent is a leukotriene modifier.

24. The method of claim 23 wherein the leukotriene modifier is selected from the group consisting of ibudilast, montelukast, pranlukast, and zafirlukast.

25. The method of claim 12 wherein the additional agent is a phosphodiesterase-4 inhibitor.

26. The method of claim 25 wherein the phosphodiesterase-4 inhibitor is selected from the group consisting of roflumilast and cilomilast.

27. The method of claim 1 wherein the disease or condition associated with the activity of a GPCR is congestive heart failure (CHF).

28. The method of claim 27 wherein the GPCR is a β_2-adrenergic receptor.

29. The method of claim 28 wherein the inverse agonist is selected from the group consisting of carvedilol and nadolol.

30. The method of claim 1 wherein the disease or condition associated with the activity of a GPCR is associated with the activity of the histamine H_1 receptor.

31. The method of claim 30 wherein the disease or condition is chronic allergic rhinitis.

32. The method of claim 30 further comprising administering an H_1 agonist.

33. The method of claim 32 wherein the H_1 agonist is histamine.

34. The method of claim 32 wherein the H_1 agonist is a histamine analogue.

35. The method of claim 1 wherein the disease or condition associated with the activity of a GPCR is associated with the activity of the histamine H_2 receptor.

36. The method of claim 35 wherein the disease or condition is selected from the group consisting of gastrointestinal reflux disease (GERD) and stomach ulcers.

37. The method of claim 35 further comprising administering an H_2 agonist.

38. The method of claim 35 wherein the H_2 inverse agonist is selected from the group consisting of cimetidine, nizatidine, ranitidine, and famotidine.

39. The method of claim 27 wherein the H_2 agonist is selected from the group consisting of histamine, dimaprit, betazole, ametazole, and arpromidine.

40. The method of claim 1 wherein the GPCR is selected from the group consisting of acetylcholine receptors, α-adrenergic receptors, serotonin (5-hydroxytryptamine) receptors, dopamine receptors, adenosine receptors, angiotensin Type II receptors, bradykinin receptors, calcitonin receptors, calcitonin gene-related receptors, cannabinoid receptors, cholecystokinin receptors, chemokine receptors, cytokine receptors, gastrin receptors, endothelin receptors, γ-aminobutyric acid (GABA) receptors, galanin receptors, glucagon receptors, glutamate receptors, luteinizing hormone receptors, choriogonadotrophin receptors, follicle-stimulating hormone receptors, thyroid-stimulating hormone receptors, gonadotrophin-releasing hormone receptors, leukotriene receptors, Neuropeptide Y receptors, opioid receptors, parathyroid hormone receptors, platelet activating factor receptors, prostanoid

(prostaglandin) receptors, somatostatin receptors, thyrotropin-releasing hormone receptors, vasopressin and oxytocin receptors.

41. The method of claim 40 further comprising administering an agonist to the GPCR.

42. A method for screening a compound for inverse agonist activity against a GCPR comprising the steps of:

(a) providing a population of specific G protein coupled receptors characterized by a constitutive basal level of activity in the absence of an agonist;

(b) contacting the population of specific G protein coupled receptors with a compound to be screened for its inverse agonist activity, the compound not being an agonist of the population of specific G protein coupled receptors; and

(c) determining the constitutive basal level of activity of the specific G protein coupled receptors in the absence of the compound and in the presence of the compound, such that the constitutive basal level of activity decreases if the compound is an inverse agonist.

43. The method of claim 42 wherein the level of activity of the specific G protein coupled receptors is determined in an intact organism.

44. The method of claim 42 wherein the level of activity of the specific G protein coupled receptors is determined in cell culture.

45. The method of claim 42 wherein the level of activity of the specific G protein coupled receptors is determined in tissue culture.

46. The method of claim 42 wherein the production or activity of a second messenger is measured.

47. The method of claim 46 wherein the second messenger is cAMP.

48. The method of claim 43 wherein a physiological consequence of receptor activation is measured.

49. The method of claim 48 wherein the physiological consequence of receptor activation is airway resistance.

50. The method of claim 42 wherein the population of specific G protein coupled receptors is provided in cells transformed or transfected with genetically engineered constitutively active mutant receptors.

51. The method of claim 42 wherein the population of specific G protein coupled receptors is provided in cells that overexpress wild-type receptors.

52. A method for screening a compound for inverse agonist activity against a GCPR comprising the steps of:

(a) providing cells containing a population of specific G protein coupled receptors characterized by a constitutive basal level of activity in the absence of an agonist;

(b) contacting the cells containing the population of specific G protein coupled receptors with a compound to be screened for its inverse agonist activity, the compound not being an agonist of the population of specific G protein coupled receptors, the compound being contacted with the cells for a period of time to result in an increase in receptor population or receptor density if the compound is an inverse agonist; and

(c) determining the receptor population or receptor density of the specific G protein coupled receptors in the cells in the absence of the

compound and in the presence of the compound, such that the receptor population or receptor density increases if the compound is an inverse agonist.

53. The method of claim 52 wherein the receptor population or receptor density is determined by an immunochemical method.

54. The method of claim 52 wherein the receptor population or receptor density is determined by binding of a radioligand with an affinity sufficiently high to bind all receptors and measuring the extent of binding.

55. A method for treating a disease or condition associated with the activity of a G protein coupled receptor (GPCR) comprising administering an inverse agonist for the GPCR to an organism with a disease or condition associated with the activity of the GPCR in a quantity and for a period that prevents the decrease in the population of GPCRs due to the presence of either exogenous or endogenous agonist, thereby producing a therapeutic effect to ameliorate the disease or condition.

ABSTRACT OF THE DISCLOSURE

The present invention describes a method for treating a disease or condition associated with the activity of a G protein coupled receptor (GPCR) comprising administering an inverse agonist for the GPCR to an organism with a disease or condition associated with the activity of the GPCR in a quantity and for a period that causes an increase in the population of spontaneously active GPCRs associated with that physiological function, thereby producing a therapeutic effect to ameliorate the disease or condition. This provides a basis for so-called "paradoxical pharmacology." These methods can be used to treat pulmonary airway diseases, including asthma, chronic allergic rhinitis, gastrointestinal reflux disease, and stomach ulcers, among other diseases and conditions. The present invention further describes a screening method for screening a compound for inverse agonist activity to a GPCR.

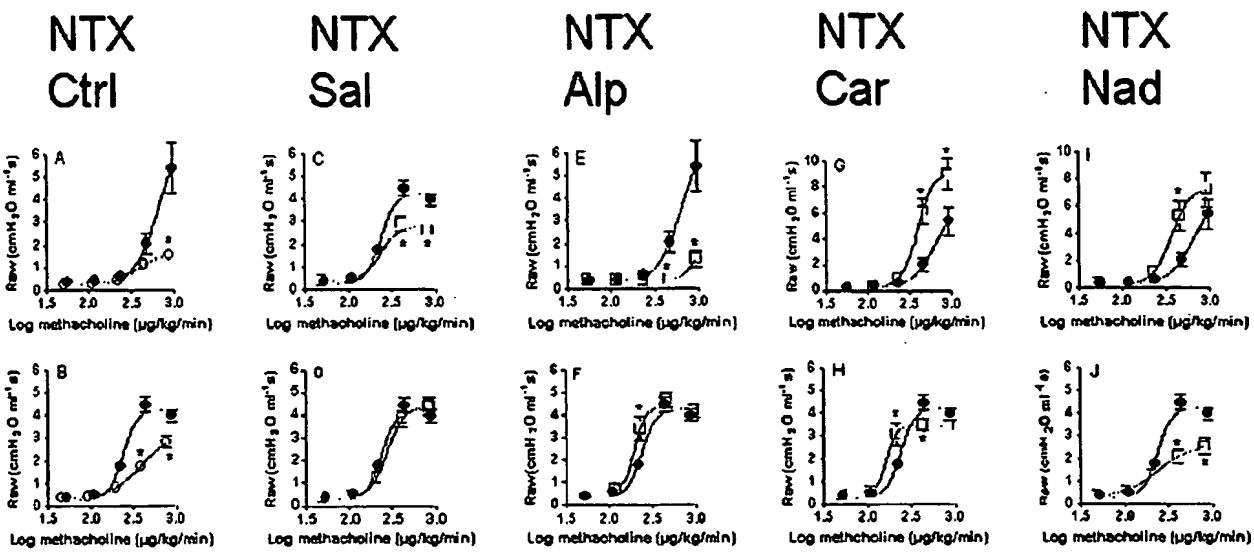
NTX Ctrl
NTX Sal
NTX Alp
NTX Car
NTX Nad
A
B
C
D
E
F
G
H
I
J
Log methacholine (µg/kg/min)

Figure 1.

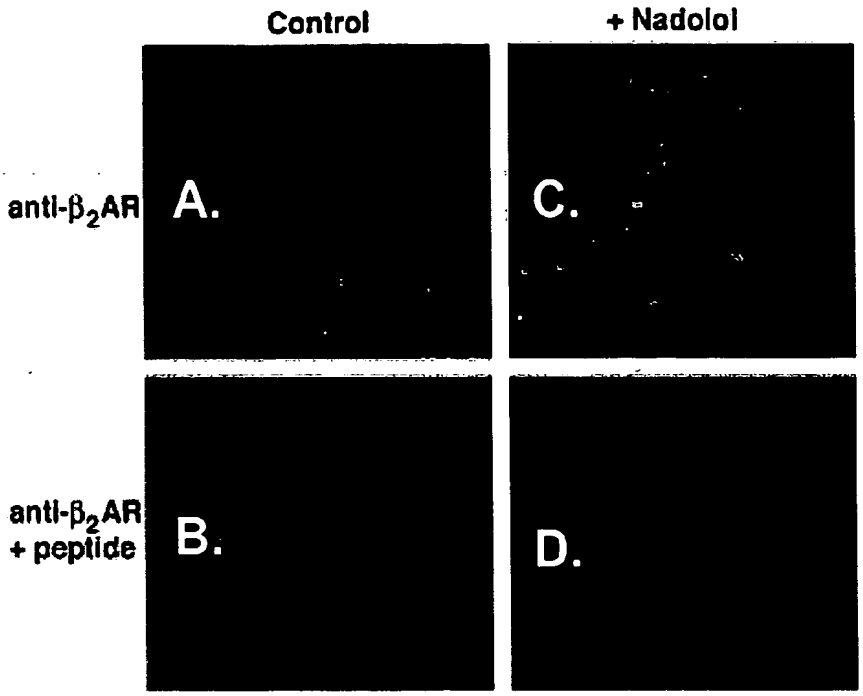

Figure 2.

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